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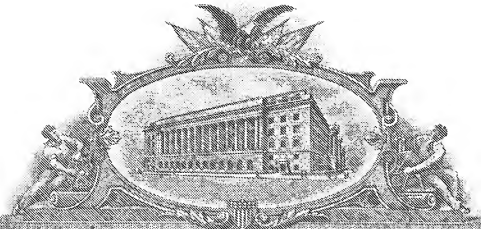

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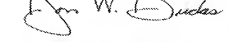

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PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53 (b)(2).

Docket Number	68666-A-Pro	Type a plus sign (+) inside this box →	+

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TITLE OF THE INVENTION (280 characters max)

COMBINATION THERAPY WITH GLATIRAMER ACETATE AND RILUZOLE FOR THE TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS

22151 U.S.PTO 60/549976 030304

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ENCLOSED APPLICATION PARTS (check all that apply)

X	Specification	Number of Pages: 32		Small Entity Statement	
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X	A check or money order is enclosed to cover the Provisional filing fees	PROVISIONAL FILING FEE AMOUNT ($)	$160.00
X	The Commissioner is hereby authorized to charge filing fees and credit Deposit Account Number: 03-3125		

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No.

[ ] Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE Gary J. Gershik Date 3/3/04

TYPED or PRINTED NAME Gary J. Gershik REGISTRATION NO. (if appropriate) 39,992

[ ] Additional inventors are being named on separately numbered sheets attached hereto

PROVISIONAL APPLICATION FILING ONLY

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Liat Hayardeny et al.

Serial No.: Not Yet Known

Filed : Herewith

For : COMBINATION THERAPY WITH GLATIRAMER ACETATE AND RILUZOLE FOR THE TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS

1185 Avenue of the Americas
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Application for United States Letters Patent

To all whom it may concern:

Be it known that, we,

Liat Hayardeny, Ety Klinger, Eran Blaugrund

have invented certain new and useful improvements in

COMBINATION THERAPY WITH GLATIRAMER ACETATE AND RILUZOLE FOR THE TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS

of which the following is a full, clear and exact description.

COMBINATION THERAPY WITH GLATIRAMER ACETATE AND RILUZOLE FOR THE TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS

Throughout this application, various events are referenced in parenthesis. Full citations for these publications may be found listed in alphabetical order at the end of the specification immediately preceding the claims. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

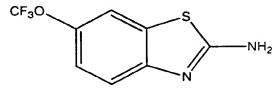
Field of the Invention

The subject invention relates to combination therapy for treating amyotrophic lateral sclerosis.

Background of the Invention

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a neurodegenerative disease that occurs when motor neurons degenerate, causing the muscles under their control to atrophy. Symptoms may include loss of motor control in one's extremities, twitching, cramping and difficulties in speaking, swallowing and breathing. Death usually occurs within 5 years of diagnosis (Amyotrophic Lateral Sclerosis Information Page, National Institute of Neurological Disorders and Stroke). The etiology and pathogenesis of ALS are not known, although a number of hypotheses have been advanced (Physician's Desk Reference, 2002). One hypothesis is that motor neurons, made vulnerable through either genetic predisposition or environmental factors, are injured by glutamate. There is evidence that mitochondrial damage and oxidative stress plays a role in human sporadic ALS (Ludolph A.C. et al.; Vielhaber S. et al.). In some cases of familial ALS, the enzyme superoxide dismutase has been found to be defective (Physician's Desk Reference, 2002).

Riluzole is a member of the benzothiazole class. Chemically, riluzole is 2-amino-6-trifluoromethoxy benzothiazole. Its molecular formula is $C_8H_5F_3N_2OS$ and its molecular weight is 234.2. Its structural formula is as follows:



and it has a molecular weight of 234.2 (Physician's Desk Reference, 2002).

RILUTEK® is a commerically available formulation of riluzole (2-amino-6-trifluoromethoxy benzothiazole), which is indicated for the treatment of patients with amyotrophic lateral sclerosis (ALS). RILUTEK® extends survival and/or time to tracheostomy. The recommended dose for RILUTEK® is 50 mg every 12 hours. RILUTEK® should be administered at least one hour before or at least two hours after a meal (Physician's Desk Reference, 2003).

PCT International Publication Nos. WO 01/52878 and WO 01/93893 list ALS as one of a number of indications which may possibly be affected by glatiramer acetate, but do not test glatiramer acetate for the treatment of ALS.

Glatiramer acetate (GA), also known as Copolymer-1, has been shown to be effective in treating multiple sclerosis (MS) (Lampert, P.W.). Daily subcutaneous injections of glatiramer acetate (20 mg/injection) reduce relapse rates, progression of disability, appearance of new lesions by magnetic resonance imaging (MRI), (Johnson, K.P. et al.) and appearance of "black holes" (Filippi, M. et al.).

COPAXONE® is the brand name for a formulation containing glatiramer acetate as the active ingredient. Glatiramer acetate is approved for reducing the frequency of relapses in relapsing-remitting multiple sclerosis. Glatiramer acetate consists of the acetate salts of synthetic polypeptides containing four naturally occurring amino acids: L-glutamic acid, L-alanine, L-tyrosine, and L-lysine with an average molar fraction in COPAXONE® of 0.141, 0.427, 0.095 and 0.338, respectively. In COPAXONE®, the average molecular weight of the glatiramer acetate is 4,700-11,000 daltons. Chemically, glatiramer acetate is designated L-glutamic acid polymer with L-alanine, L-lysine and L-tyrosine, acetate (salt). Its structural formula is:

$$(Glu, Ala, Lys, Tyr)_x \cdot CH_3COOH$$
$$(C_5H_9NO_4 \cdot C_3H_7NO_2 \cdot C_6H_{14}N_2O_2 \cdot C_9H_{11}NO_3)_x \cdot XC_2H_4O_2$$

CAS - 147245-92-9.

The recommended dosing schedule of COPAXONE® for relapsing-remitting multiple sclerosis is 20 mg per day injected subcutaneously (Physician's Desk Reference, 2003; see also U.S. Patent Nos. 3,849,550; 5,800,808; 5,858,964, 5,981,589; 6,048,898; 6,054,430; 6,214,791; 6,342,476; and 6,362,161, all of which are hereby incorporated by reference).

The administration of two drugs to treat a given condition, such amyotrophic lateral sclerosis, raises a number of potential problems. *In vivo* interactions between two drugs are complex. The effects of any single drug are related to its absorption, distribution, and elimination. When two drugs are introduced into the body, each drug can affect the absorption, distribution, and elimination of the other and hence, alter the effects of the other. For instance, one drug may inhibit, activate or induce the production of enzymes involved in a metabolic route of elimination of the other drug (Guidance for Industry. *In vivo* drug metabolism/drug interaction studies -

study design, data analysis, and recommendations for dosing and labeling). Thus, when two drugs are administered to treat the same condition, it is unpredictable whether each will complement, have no effect on, or interfere with, the therapeutic activity of the other in a human subject.

Not only may the interaction between two drugs affect the intended therapeutic activity of each drug, but the interaction may increase the levels of toxic metabolites (Guidance for Industry. *In vivo* drug metabolism/drug interaction studies - study design, data analysis, and recommendations for dosing and labeling). The interaction may also heighten or lessen the side effects of each drug. Hence, upon administration of two drugs to treat a disease, it is unpredictable what change will occur in the negative side profile of each drug.

Additionally, it is accurately difficult to predict when the effects of the interaction between the two drugs will become manifest. For example, metabolic interactions between drugs may become apparent upon the initial administration of the second drug, after the two have reached a steady-state concentration or upon discontinuation of one of the drugs (Guidance for Industry. *In vivo* drug metabolism/drug interaction studies - study design, data analysis, and recommendations for dosing and labeling).

Thus, the success of one drug or each drug alone in an *in vitro* model, an animal model, or in humans, may not correlate into efficacy when both drugs are administered to humans.

In accordance with the subject invention, glatiramer acetate and 2-amino-6-trifluoromethoxybenzathiazole are effective in combination to treat aymotrophic lateral sclerosis.

Summary of the Invention

The subject invention provides a method of treating a subject afflicted with amyotrophic lateral sclerosis comprising periodically administering to the subject an amount of glatiramer acetate and an amount of 2-amino-6-trifluoromethoxybenzathiazole, wherein the amounts when taken together are effective to alleviate a symptom of amyotrophic lateral sclerosis in the subject so as to thereby treat the subject.

In addition, the subject invention provides a package comprising

i) a first pharmaceutical composition comprising an amount of glatiramer acetate and a pharmaceutically acceptable carrier;

ii) a second pharmaceutical composition comprising an amount of 2-amino-6-triflurormethoxybenzothiazole and a pharmaceutically acceptable carrier; and

iii) instructions for use of the first and second pharmaceutical compositions together to alleviate a symptom of amyotrophic lateral sclerosis in a subject.

The subject invention further provides a pharmaceutical composition comprising an amount of glatiramer acetate and an amount of 2-amino-6-triflurormethoxybenzothiazole, wherein the amounts when taken together are effective to alleviate a symptom of amyotrophic lateral sclerosis in a subject.

Detailed Description of the Invention

The subject invention provides a method of treating a subject afflicted with amyotrophic lateral sclerosis comprising periodically administering to the subject an amount of glatiramer acetate and an amount of 2-amino-6-trifluoromethoxybenzathiazole, wherein the amounts when taken together are effective to alleviate a symptom of amyotrophic lateral sclerosis in the subject so as to thereby treat the subject.

In one embodiment, the subject is a human being.

In a further embodiment, each of the amount of glatiramer acetate when taken alone, and the amount of 2-amino-6-trifluoromethoxybenzathiazole when taken alone is effective to alleviate the symptom of amyotrophic lateral sclerosis.

In an embodiment, either the amount of glatiramer acetate when taken alone, the amount of 2-amino-6-trifluoromethoxybenzathiazole when taken alone or each such amount when taken alone is not effective to alleviate the symptom of amyotrophic lateral sclerosis.

In yet another embodiment, the symptom is twitching, cramping, loss of motor control, or difficulties in speaking, swallowing or breathing.

In one embodiment, the amount of glatiramer acetate may be 10 to 80 mg; or 12 to 70 mg; or 14 to 60 mg; or 16 to 50 mg; or 18 to 40 mg; or 20 to 30 mg; or 20 mg. For each amount of glatiramer acetate, the amount of 2-amino-6-trifluoromethoxybenzathiazole may be 25 to 75 mg; or 35 to 65 mg; or 45 to 55 mg; or 50 mg.

Alternatively, the amount of glatiramer acetate may be in the range from 10 to 600 mg/week; or 100 to 550 mg/week; or 150 to 500 mg/week; or 200 to 450 mg/week; or 250 to 400 mg/week; or 300 to 350 mg/week; or 300 mg/week.

In another embodiment, the amount of glatiramer acetate may be in the range from 50 to 150 mg/day; or 60 to 140 mg/day; or 70 to 130 mg/day; or 80 to 120 mg/day; or 90 to 110 mg/day; or 100 mg/day.

Alternatively, the amount of glatiramer acetate may be in the range from 10 to 80 mg/day; or 12 to 70 mg/day; or 14 to 60 mg/day; or 16 to 50 mg/day; or 18 to 40 mg/day; or 19 to 30 mg/day; or 20 mg/day.

In one embodiment, the periodic administration of glatiramer acetate is effected daily.

In another embodiment, the periodic administration of glatiramer acetate is effected twice daily at one half the amount.

In an additional embodiment, the periodic administration of glatiramer acetate is effected once every 3 to 11 days; or once every 5 to 9 days; or once every 7 days; or once every 24 hours.

For each administration schedule of glatiramer acetate, the 2-amino-6-trifluoromethoxybenzathiazole may be administered once every 8 to 16 hours; or once every 10 to 14 hours; or once every 12 hours.

In an embodiment, the periodic administration of 2-amino-6-trifluoromethoxybenzathiazole is effected at least one hours before or at least two hours after a meal.

In a further embodiment, the administration of the glatiramer

acetate substantially precedes the administration of the 2-amino-6-trifluoromethoxybenzathiazole.

In an added embodiment, the administration of the 2-amino-6-trifluoromethoxybenzathiazole substantially precedes the administration of the glatiramer acetate.

In one embodiment, the glatiramer acetate and the 2-amino-6-trifluoromethoxybenzathiazole may be administered for a period of time of at least 4 days. In a further embodiment, the period of time may be 5 days to 5 years; or 10 days to 3 years; or 2 weeks to 1 year; or 1 month to 6 months; or 3 months to 4 months. In yet another embodiment, the glatiramer acetate and the 2-amino-6-trifluoromethoxybenzathiazole may be administered for the lifetime of the subject.

The administration of 2-amino-6-trifluoromethoxybenzathiazole or glatiramer acetate may each independently be oral, nasal, pulmonary, parenteral, intravenous, intra-articular, transdermal, intradermal, subcutaneous, topical, intramuscular, rectal, intrathecal, intraocular, buccal or by gavage. For 2-amino-6-trifluoromethoxybenzathiazole, the preferred route of administration is oral or by gavage. The preferred route of administration for glatiramer acetate is subcutaneous or oral. One of skill in the art would recognize that doses at the higher end of the range may be required for oral administration.

In one embodiment, the administration of the glatiramer acetate may be subcutaneous, intraperitoneal, intravenous, intramuscular, intraocular or oral and the administration of the 2-amino-6-trifluoromethoxybenzathiazole may be oral. In another embodiment, the administration of the glatiramer acetate may be subcutaneous and the administration of the 2-amino-6-trifluoromethoxybenzathiazole may be oral.

The subject invention also provides a package comprising

i) a first pharmaceutical composition comprising an amount of glatiramer acetate and a pharmaceutically acceptable carrier;

ii) a second pharmaceutical composition comprising an amount of 2-amino-6-triflurormethoxybenzothiazole and a pharmaceutically acceptable carrier; and

iii) instructions for use of the first and second pharmaceutical compositions together to alleviate a symptom of amyotrophic lateral sclerosis in a subject.

In an embodiment of the package, the amount of glatiramer acetate may be in the range from 10 to 600 mg; or 100 to 550 mg; or 150 to 500 mg; or 200 to 450 mg; or 250 to 400 mg; or 300 to 350 mg; or 300 mg.

In another embodiment of the package, the amount of glatiramer acetate may be in the range from 10 to 80 mg; or 12 to 70 mg; or 14 to 60 mg; or 16 to 50 mg; or 18 to 40 mg; or 19 to 30 mg; or 20 mg.

Alternatively, the amount of glatiramer acetate in the package may be in the range from 50 to 150 mg; or 60 to 140 mg; or 70 to 130 mg; or 80 to 120 mg; or 90 to 110 mg; or 100 mg.

For each amount of glatiramer acetate in the package, the amount of 2-amino-6-trifluoromethoxybenzathiazole in the package may be 25-75 mg; or 35-65 mg; or 45-55 mg; or 50 mg.

The subject invention further provides a pharmaceutical composition comprising an amount of glatiramer acetate and an amount of 2-amino-6-triflurormethoxybenzothiazole, wherein the amounts when taken together are effective to alleviate a symptom of amyotrophic lateral sclerosis in a subject.

In one embodiment of the pharmaceutical composition, each of the amount of glatiramer acetate when taken alone and the amount of 2-amino-6-trifluoromethoxybenzathiazole when taken alone is effective to alleviate the symptom of amyotrophic lateral sclerosis.

In another embodiment of the pharmaceutical composition, either of the amount of glatiramer acetate when taken alone, or the amount of 2-amino-6-trifluoromethoxybenzathiazole when taken alone or each such amount when taken alone is not effective to alleviate the symptom of amyotrophic lateral sclerosis.

The subject invention further provides a pharmaceutical combination comprising separate dosage forms of an amount of glatiramer acetate and an amount of 2-amino-6-triflurormethoxybenzothiazole, which combination is useful to alleviate a symptom of amyotrophic lateral sclerosis in a subject.

In an embodiment of the pharmaceutical combination, each of the amount of glatiramer acetate when taken alone and the amount of 2-amino-6-trifluoromethoxybenzathiazole when taken alone is effective to alleviate the symptom of amyotrophic lateral sclerosis.

In an additional embodiment of the pharmaceutical combination, wherein either of the amount of glatiramer acetate when taken alone, the amount of 2-amino-6-trifluoromethoxybenzathiazole when taken alone or each such amount when taken alone is not effective to alleviate the symptom of amyotrophic lateral sclerosis.

In a further embodiment, the pharmaceutical combination may be for simultaneous, separate or sequential use to treat amyotrophic lateral sclerosis in the subject.

Formulations of the invention suitable for oral administration may be in the form of capsules, pills, tablets, powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of the active compound or compounds.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient(s) is mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; humectants, such as glycerol; disintegrating agents, such as agar-agar, calcium carbonate, calcium phosphate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; solution retarding agents, such as paraffin; absorption accelerators, such as quaternary ammonium compounds; wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; absorbents, such as kaolin and bentonite clay; lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

Liquid dosage forms for oral administration of the active ingredients include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient(s), the liquid dosage forms may contain inert dilutents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Suspensions, in addition to the active compounds, may contain suspending agents such as ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

The pharmaceutical compositions, particularly those comprising glatiramer acetate, may also include human adjuvants or carriers known to those skilled in the art. Such adjuvants include complete Freund's adjuvant and incomplete Freund's adjuvant. The compositions may also comprise wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Glatiramer acetate may be formulated into pharmaceutical compositions with pharmaceutically acceptable carriers, such as water or saline and may be formulated into eye drops. Glatiramer acetate may also be formulated into delivery systems, such as matrix systems.

This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will

readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

Experimental Details

EXAMPLE 1: GLUTAMATE TOXICITY

Under normal circumstances, glutamate functions as an essential neurotransmitter. However, when glutamate levels rise above the normal level, glutamate becomes toxic. Elevated glutamate levels and the resultant toxicity are implicated in many diseases, as discussed in the Background of the Invention.

Procedure

60 mice are injected with glutamate (0.2 M) to induce retinal ganglion cell (RGC) death. As shown in Table 1, mice are immunized with glatiramer acetate, riluzole or both prior to glutamate injection. Glatiramer acetate is given s.c. (subcutaneously), 100 µl/mouse, with or without adjuvant. Glatiramer acetate can also be administered orally, with or without adjuvant. Glatiramer acetate may be administered over several doses before the glutamate challenge or may be administered simultaneously with glutamate. Riluzole is administered by gavage in 4 doses of 10 mg/kg each. The control animals receive PBS without any active agents, although any vehicle can be used.

Table 1: Administration Protocol

Experimental Groups	Glatiramer Acetate	Riluzole	Glutamate
A	X	X	X
B	X	-	X
C	-	X	X
D	-	-	X
E			

7 days after glutamate injection, mice are sacrificed, retinas are excised and surviving RGCs are counted. Following immunization, *in vitro* cellular response to specific activators,

such as glatiramer acetate, and to non-specific activators, such as Con-A, PHA, etc., are assessed.

Results

The administration of only glatiramer acetate (Group B) or only riluzole (Group C) prior to glutamate challenge raises the number of surviving RGCs above that of the control group that receives only glutamate (Group E). When both glatiramer acetate and riluzole are administered before the glutamate administration (Group A), the number of surviving RGCs is comparable to or greater than the number of surviving RGCs in Group B (glatiramer acetate alone) or Group C (riluzole alone).

EXAMPLE 2: MPTP-INDUCED DOPAMINERGIC NEUROTOXICITY

MPTP is a neurotoxin that damages nigrostriatal dopaminergic neurons in several mammalian species, including mice, and produces a Parkinsonian syndrome in humans and primates. A crucial initial step in the mechanism of its neurotoxicity involves conversion of MPTP to its toxic metabolite 1-methyl-4-phenyl pyridinium ion (MPP+). This reaction is catalyzed by the enzyme MAO-B and probably takes place outside of dopaminergic neurons, mainly in glia (U.S. Patent No. 6,316,504).

A) Methodology

i) Animals

Mice (C57Bl6 males weighing 20-25 g, 6-8 weeks of age) are obtained from Harlan (Jerusalem) and housed 5 per cage for 1 week before treatment. Standard mouse chow and water is supplied *ad libitum*. Room lighting is 12 hours light, 12 hours dark; lights on at 7:00 AM. The cages are maintained in a locked room in the animal house, accessible only to personnel familiar with the safety rules for MPTP administration, and wearing appropriate protective clothing.

ii) Materials

Glatiramer acetate

Riluzole

MPTP hydrochloride, Sigma cat. # M0896, lot #89H4702

PBS: phosphate-buffered saline (g per liter): NaCl 8, KCl 0.2, Na_2HPO_4 1.44, KH_2PO_4 0.24; pH 7.4 (materials from Sigma, Israel)

sterile saline: NaCl, 0.9%

iii) Glatiramer acetate/riluzole administration

Table 2 presents the dosing schedule for the groups of mice (10 mice per group). 7 days before MPTP administration, the mice in Groups B, D and F are injected s.c. with 0.2 ml of a solution of glatiramer acetate (0.5 mg/ml) in PBS. This injection correlates to 100 µg/mouse. This dose is used because it has been used in other studies (glutamate toxicity, retinal intraocular pressure (IOP) model). Riluzole (10 mg/kg) is administered by gavage to the mice in Groups B, E and F 30 minutes before each of 4 MPTP injections. The members of control group A are injected s.c. with 0.2 ml of PBS and do not receive MPTP. The mice in control group B are injected s.c. with glatiramer acetate (100 µg/mouse) and given riluzole (10 mg/kg) by gavage, and saline instead of MPTP. The mice in control group C are injected s.c. with 0.2 ml of PBS followed by MPTP administration as outlined below.

Table 2: Administration Protocol

Experimental Groups	**GA**	**Riluzole**	**MPTP**
A			
B	X	X	
C			X
D	X		X
E		X	X
F	X	X	X

iv) MPTP administration

For the mice that have received glatiramer acetate, 7 days after glatiramer acetate immunization, cage bedding is changed and MPTP treatment is commenced. MPTP is obtained from Sigma in the form of 10 mg pre-weighed vials. The vial contents are dissolved in 2 ml sterile saline solution (0.9%) to yield a solution containing 5 mg/ml MPTP. Each mouse is injected

intraperitoneally (i.p.) with 0.1 ml of this solution per 20 g body weight, to give a dose of 25 mg per kg body weight. A total of 4 injections are given to each mouse, on each of 4 successive days, injections being made between 10 AM and 12 noon. Control animals receive the same volume of sterile saline (0.1 ml per 20 g body weight).

v) Precautions during use of MPTP

Personnel wear disposable laboratory coats, disposable latex rubber gloves and carbon filter face masks for the administration of MPTP. Cage bedding and all utensils in contact with MPTP is exposed to a 20% Clorox® solution (commercial bleach) for 30 minutes before containment in biohazard grade disposable nylon bags.

vi) Clinical assessment

Mice are observed for 30 minutes following each injection of MPTP.

vii) Determination of striatal dopamine

Mice are sacrificed by cervical dislocation 7 days after the first MPTP injection. Brains are removed and cooled on ice. Each whole brain is placed on its dorsal surface on an ice-cooled glass plate. A coronal cut is made with a razor blade at the level of the optic chiasm. Striata is dissected from the frontal part of the brain and snap-frozen in liquid nitrogen, then stored at -70°C until homogenization.

Immediately prior to homogenization, the tissue is weighed. The vials are removed from the -70°C refrigerator and quickly placed in a liquid nitrogen container. Taking care to keep the cap closed, each vial is then separately removed from the liquid nitrogen container. The frozen tissue is then quickly and carefully removed from the vial and placed on a pre-weighed dish in the analytical balance. The tissue is then weighed.

For homogenization, the tissue is placed in an Ependorff tube to which 300 µl of a 0.1 M perchlorate solution containing 2 mM sodium metabisulfite and 0.25 mM ethylenediaminetetracetic acid (EDTA) is added. The tissue is homogenized for 30 seconds in ice using an Ependorff miniature homogenizer. Then, 300 µl perchlorate is added (0.6 ml for more than 20 mg tissue).

The tissue is then centrifuged at 13000 g for 5-7 minutes and the supernatant is decanted for catecholamine determination. If the analysis is not done on the same day, the samples can be frozen at -70°C and centrifuged again after thawing before analysis.

To detect the presence and amounts of dopamine, DOPAC and HVA, a hypersil H3ODS column (packing 3 mm, 4.6 mm diameter, 12.5 cm long) is used. The mobile phase is composed of NaH_2PO_4 100 mM, octan-1-sulphonic acid 1.5 mM, disodium ethylenediaminetetracetic acid 250 µM, methanol 2.3%, and acetonitrile 4% in HPLC grade deionized water, at a flow rate of 1.0 ml min^{-1}. Compounds are detected with an ESA Coulochem model 5014 electrochemical detector (Bedford, MA, USA). Column eluates are initially oxidized by an ESA guard cell (model 5020) at +300 mV, then reduced at +60 mV at detector 1 and measured at detector 2 at -350 mV. The catecholamines are compared to standards (10^{-7}M prepared in perchlorate). Dilution of the sample (1:10) for the dopamine determination might be needed. Levels of dopamine and metabolites are expressed in terms of pmol per mg tissue (frozen weight).

B) Results

In the absence of MPTP (Groups A and B), striatal dopamine levels are around 60-90 pmol/mg tissue. The administration of MPTP reduces the striatal dopamine levels to around 25-45 pmol/mg tissue (Group C). Immunization with glatiramer acetate alone (Group D) prior to the administration of MPTP increases the striatal dopamine levels. The administration of riluzole alone (Group E) before MPTP challenge increases the striatal dopamine levels. In Group F, the combined administration of glatiramer acetate and riluzole before injection of MPTP results striatal dopamine levels that are comparable to or greater than the striatal dopamine levels of Group D (glatiramer acetate alone) or Group E (riluzole alone).

EXAMPLE 3: EXPERIMENTAL MODEL FOR AMYOTROPHIC LATERAL SCLEROSIS

Procedure

Transgenic mice carrying multiple copies of the human G93A Cu/Zn SOD mutation are considered to be the best model system for anterior horn cell degenerations such as amyotrophic lateral sclerosis (Ludolph et al.; Gurney et al. 1994 and 1996).

i) Animals

Transgenic mice overexpressing human Cu/Zn-SOD G93A mutations ((B6SJL-TgN (SOD1-G93A) 1 Gur) and non-transgenic B6/SJL mice are purchased from Jackson Laboratories (Bar Harbor, ME, USA). The second generation of G1H mice are used.

ii) Treatment Protocol

The SOD1 transgenic mice are treated in five groups (N = 15) with two dosages of glatiramer acetate alone or in combination with riluzole. A group of 15 mice serve as controls.

Treatment protocols for the six groups were the following:

Group I: Low dose glatiramer acetate

Group II: High dose glatiramer acetate

Group III: Riluzole 30 mg /kg per day

Group IV: Low dose glatiramer acetate and riluzole 30 mg/kg per day

Group V: High dose glatiramer acetate 2.0 mg/kg and riluzole 30 mg/kg per day

Group VI: Controls (placebo)

The drugs are administered in drinking water starting at 60 days of age.

iii) Survival

The clinical condition of the mice is monitored daily starting at 40 days. The onset of clinical signs is scored by examining the mice for tremors and/or shaking of the limbs, the position of one or both hind limbs (hanging rather than splaying out) when the mice are suspended in the air by their tail. The age of clinical onset was determined by the age (days) at which loss of splay or tremors of hind limbs is observed. The loss of righting reflex determines the end stage of the disease. The mice are sacrificed if they could not right themselves within 30 seconds when placed on either side on a flat surface.

iv) Behavior and Weight Assessment

Mice are observed daily (including weekends) and weighed weekly. Motor performance is assessed from 40 days of age using the rotarod apparatus to measure the night activity of the mice from 8 p.m. - 8 a.m. (LMTB, Berlin).

v) Neuropathological Studies

Mice were perfused transcardially with 4% paraformaldehyde/0.9% NaCl solution, brains and whole spinal cord were dissected out, frozen in liquid nitrogen and cut in transverse sections of 20 µm on a sliding microtome. Sections of brainstem and spinal cord were stained with HE, toluidine blue (semi-thin sections, 0.5 µm), and immunohistochemistry to label astrocytes (GFAP), cholinergic motoneurons (ChAT) and dopaminergic cells (TH).

Results

i) Survival

The primary end point of this study is survival. Animals treated with the low dose of glatiramer acetate alone show an increse in life span which is not statistically significant. The high dose of glatiramer acetate produces a statistically significant increase in life span. Both combinations of

riluzole and glatiramer acetate result in an increase in life span which is comparable to or greater than the increase in life span in the groups receiving only glatiramer acetate.

ii) Behavior

The results of measurements of running wheel activity in the 6 groups are largely complementary to the survival data. Statistical analysis show that glatiramer acetate-treated (at both dosages) and riluzole-treated animals are more active during the course of treatment and maintain their motor activity longer than controls. Treatment with riluzole combined with either low-dose or high-dose glatiramer acetate results in activity comparable to or greater than treatment with riluzole alone or glatiramer acetate alone.

iii) Neuropathological Studies

Neuropathological examination of the spinal cord shows the typical features of G93A-associated damage; i.e. loss of motor neurons, astrocytosis and extensive vacuolation of the spinal grey matter. At the light-microscopic level, there are no differences in the degree of neuronal loss, the morphology of astrocytosis or the extent of vacuolisation between animals. This lack of effect on histopathology may be due to the fact that all the animals are sacrificed at terminal stages of the disease.

References

U.S. Patent No. 3,849,550, issued November 19, 1974 (Teitelbaum, et al.).

U.S. Patent No. 5,486,541, issued January 23, 1996 (Sterling et al.).

U.S. Patent No. 5,674,885, issued October 7, 1997 (Boireau et al.).

U.S. Patent No. 5,800,808, issued September 1, 1998 (Konfino, et al.).

U.S. Patent No. 5,858,964, issued January 12, 1999 (Aharoni, et al.).

U.S. Patent No. 5,981,589, issued November 9, 1999 (Konfino, et al.).

U.S. Patent No. 6,048,898, issued April 11, 2000 (Konfino, et al.).

U.S. Patent No. 6,054,430, issued April 25, 2000 (Konfino, et al.).

U.S. Patent No. 6,214,791, issued April 10, 2001 (Arnon, et al.).

U.S. Patent No. 6,316,504, issued November 13, 2002 (Youdim et al.).

U.S. Patent No. 6,342,476, issued January 29, 2002 (Konfino, et al.).

U.S. Patent No. 6,362,161, issued March 26, 2002 (Konfino et al.).

U.S. Patent No. 6,395,546, issued May 28, 2002 (Zobel et al.).

PCT International Publication No. WO 01/52878 (PCT/US01/02117), published July 26, 2001 (Eisenbach-Schwartz et al.).

PCT International Publication No. WO 01/93893 (PCT/US01/02118), published December 13, 2001 (Eisenbach-Schwartz et al.).

Filippi, M. et al., Glatiramer acetate reduces the proportion of MS lesions evolving into black holes, *Neurol.*, 2001, 57:731-733.

Gurney et al. Benefit of vitamin E, riluzole, and gabapentin in a transgenic model of familial amyotrophic lateral sclerosis. *Ann. Neurol.* 1996, 39: 147-157.

Gurney et al. Motor neuron degeneration in mice that express a human superoxide dismutase mutation. *Science* 1994, 264: 1772-1775.

Johnson, K.P. et al. Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double-blind placebo-controlled trial. The Copolymer 1 Multiple Sclerosis Study Group. *Neurol.*, 1995, 45:1268.

Lampert, P.W., Autoimmune and virus-induced demyelinating diseases. A review, 1978, *Am. J. Path.* 91:17.

Ludolph A.C., Meyer T., Riepe M.W., Antiglutamate therapy in ALS - which is the next step?, *J. Neural Transm.* 1999, 55 (Suppl.):79-96, 6-208.

Vielhaber S. et al., Mitochondrial DNA abnormalities in skeletal muscle of patients with sporadic amyotrophic lateral sclerosis, *Brain*, 2000, 123:1339-1348.

Amyotrophic Lateral Sclerosis Information Page, National Institute of Neurological Disorders and Stroke, July 1, 2001 <http://www.ninds.nih.gov/health_and_medical/disorders/amyotrophiclateralscelerosis_doc.html>.

"COPAXONE®" in Physician's Desk Reference, Medical Economics Co., Inc., Montvale, NJ, 2003, 3214-3218.

Guidance for Industry. *In vivo* drug metabolism/drug interaction studies - study design, data analysis, and recommendations for dosing and labeling, U.S. Dept. Health and Human Svcs., FDA, Ctr. for Drug Eval. and Res., Ctr. for Biologics Eval. and Res., Clin. / Pharm., Nov. 1999 <http://www.fda.gov/cber/gdlns/metabol.pdf>.

"RILUTEK®" in Physician's Desk Reference, Medical Economics Co., Inc., Montvale, NJ, 2003, 767-769.

What is Claimed:

1. A method of treating a subject afflicted with amyotrophic lateral sclerosis comprising periodically administering to the subject an amount of glatiramer acetate and an amount of 2-amino-6-trifluoromethoxybenzathiazole, wherein the amounts when taken together are effective to alleviate a symptom of amyotrophic lateral sclerosis in the subject so as to thereby treat the subject.

2. The method of claim 1, wherein the subject is a human being.

3. The method of claim 1, wherein each of the amount of glatiramer acetate when taken alone, and the amount of 2-amino-6-trifluoromethoxybenzathiazole when taken alone is effective to alleviate the symptom of amyotrophic lateral sclerosis.

4. The method of claim 1, wherein either the amount of glatiramer acetate when taken alone, the amount of 2-amino-6-trifluoromethoxybenzathiazole when taken alone or each such amount when taken alone is not effective to alleviate the symptom of amyotrophic lateral sclerosis.

5. The method of claim 1, wherein the symptom is twitching, cramping, loss of motor control, or difficulties in speaking, swallowing or breathing.

6. The method of claim 1, wherein the amount of glatiramer acetate is in the range from 10 to 600 mg/week.

7. The method of claim 6, wherein the amount of glatiramer acetate is 300 mg/week.

8. The method of claim 1, wherein the amount of glatiramer acetate is in the range from 50 to 150 mg/day.

9. The method of claim 8, wherein the amount of glatiramer acetate is 100 mg/day.

10. The method of claim 1, wherein the amount of glatiramer acetate is in the range from 10 to 80 mg/day.

11. The method of claim 10, wherein the amount of glatiramer acetate is 20 mg/day.

12. The method of claim 1, wherein the periodic administration of glatiramer acetate is effected daily.

13. The method of claim 1, wherein the periodic administration of glatiramer acetate is effected twice daily at one half the amount.

14. The method of claim 1, wherein the periodic administration of glatiramer acetate is effected once every 5 to 9 days.

15. The method of claim 1, wherein the periodic administration of 2-amino-6-trifluoromethoxybenzathiazole is effected at least one hours before or at least two hours after a meal.

16. The method of claim 1, wherein the administration of the glatiramer acetate substantially precedes the administration of the 2-amino-6-trifluoromethoxybenzathiazole.

17. The method of claim 1, wherein the administration of the 2-amino-6-trifluoromethoxybenzathiazole substantially precedes the administration of the glatiramer acetate.

18. The method of claim 1, wherein the administration of the glatiramer acetate is effected subcutaneously, intraperitoneally, intravenously, intramuscularly, intraocularly or orally and the administration of the 2-amino-6-

trifluoromethoxybenzathiazole is effected orally.

19. The method of claim 18, wherein the administration of the glatiramer acetate is effected subcutaneously and the administration of the 2-amino-6-trifluoromethoxybenzathiazole is effected orally.

20. A package comprising

iv) a first pharmaceutical composition comprising an amount of glatiramer acetate and a pharmaceutically acceptable carrier;

v) a second pharmaceutical composition comprising an amount of 2-amino-6-triflurormethoxybenzothiazole and a pharmaceutically acceptable carrier; and

vi) instructions for use of the first and second pharmaceutical compositions together to alleviate a symptom of amyotrophic lateral sclerosis in a subject.

21. The package of claim 20, wherein the amount of glatiramer acetate is 300 mg.

22. The package of claim 20, wherein the amount of glatiramer acetate is 20 mg.

23. A pharmaceutical composition comprising an amount of glatiramer acetate and an amount of 2-amino-6-triflurormethoxybenzothiazole, wherein the amounts when taken together are effective to alleviate a symptom of amyotrophic lateral sclerosis in a subject.

24. The pharmaceutical composition of claim 23, wherein each of the amount of glatiramer acetate when taken alone and the amount of 2-amino-6-trifluoromethoxybenzathiazole when taken alone is effective to alleviate the symptom of amyotrophic lateral sclerosis.

25. The pharmaceutical composition of claim 23, wherein either of the amount of glatiramer acetate when taken alone, or the amount of 2-amino-6-trifluoromethoxybenzathiazole when taken alone or each such amount when taken alone is not effective to alleviate the symptom of amyotrophic lateral sclerosis.

26. A product containing glatiramer acetate and 2-amino-6-trifluoromethoxybenzathiazole as a combined preparation for simultaneous, separate or sequential use in therapy.

27. A product containing glatiramer acetate and 2-amino-6-trifluoromethoxybenzathiazole as a combined preparation for simultaneous, separate or sequential use in therapy of amyotrophic lateral sclerosis.

28. The use glatiramer acetate and 2-amino-6-trifluoromethoxybenzathiazole for the manufacture of a combined perparation medicament for the treatment of amyotrophic lateral sclerosis, wherein glatiramer acetate and 2-amino-6-trifluoromethoxybenzathiazole are administered simultaneously, separately or sequentially.

29. The product or use of any one of claims 26-28, wherein the use is sequential at an interval of up to 24 hours.

30. The product or use of claims 29, wherein the interval is from 1 to 12 hours.

31. The product or use of claims 30, wherein the interval is 2 hours.

32. The product or use of any one of claims 26-28, wherein the use

is separate.

33. The product or use of any one of claims 26-28, wherein the use is simultaneous.

34. The use 2-amino-6-trifluoromethoxybenzathiazole for the manufacture of a medicament for the treatment of amyotrophic lateral sclerosis in a patient who is being treated with glatiramer acetate for the treatment of amyotrophic lateral sclerosis.

35. The use 2-amino-6-trifluoromethoxybenzathiazole for the manufacture of a medicament for the treatment of amyotrophic lateral sclerosis in a patient population that is being treated with glatiramer acetate for the treatment of amyotrophic lateral sclerosis.

36. The use 2-amino-6-trifluoromethoxybenzathiazole for the manufacture of a medicament for enhancing the treatment of amyotrophic lateral sclerosis in a patient who is being treated with glatiramer acetate for the treatment of amyotrophic lateral sclerosis.

COMBINATION THERAPY WITH GLATIRAMER ACETATE AND RILUZOLE FOR THE TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS

Abstract of the Disclosure

The subject invention provides a method of treating a subject afflicted with amyotrophic lateral sclerosis comprising periodically administering to the subject an amount of glatiramer acetate and an amount of 2-amino-6-trifluoromethoxybenzathiazole, wherein the amounts when taken together are effective to alleviate a symptom of the amyotrophic lateral sclerosis in the subject so as to thereby treat the subject. The subject invention also provides a package comprising glatiramer acetate, 2-amino-6-triflurormethoxybenzothiazole and instructions for use together to alleviate a symptom of amyotrophic lateral in a subject. Additionally, the subject invention provides a pharmaceutical composition comprising an amount of glatiramer acetate and an amount of 2-amino-6-triflurormethoxybenzothiazole, wherein the amounts when taken together are effective to alleviate a symptom of amyotrophic lateral sclerosis in a subject. The subject invention further provides a pharmaceutical combination comprising separate dosage forms of an amount of glatiramer acetate and an amount of 2-amino-6-triflurormethoxybenzothiazole, which combination is useful to alleviate a symptom of amyotrophic lateral sclerosis in a subject.